

COMMERCIAL INSTRUMENTS

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Commercial Instruments \*

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## I. Introduction

This chapter is intended to provide an overview of the variety of flow instruments available commercially. Characteristics of the various instruments are presented in tabular form, grouped by instrument function.

The term flow cytometer includes a large array of devices, many of them having nothing to do with analytical cytology. Many kinds of flow cytometers have been manufactured by many companies for over 30 years, many of them available for only a short time. This chapter will discuss only those instruments that are commercially available at the time of this writing, as reported to the author by the manufacturers. Some instruments that have been advertised, particularly at international conferences, are not reported on here due to lack of information from their manufacturers. Other instruments will probably come into existence by the time this book is published.

There are two major classes of instruments: flow cytometers and cell sorters. A flow cytometer is defined as a device that measures components and properties of cells and cell organelles that are suspended in a flowing liquid suspension. Cell sorters have the same capabilities as flow cytometers with the additional capability of selectively removing objects (cells, chromosomes, etc.) from the liquid suspension. Both classes of instruments operate at high measurement rates, typically several thousand per second. The tables that follow present these two classes separately. These tables are standardized to show the same information for each instrument. More information may be available directly from the manufacturers. Also, data are reported only for complete systems. There are several manufacturers that produce parts of a system, notably the data analysis systems. These manufacturers are listed in Section III.

It is hoped that users and potential users of flow cytometers and cell sorters will find the information presented here useful both in selecting new instruments and in upgrading current ones.

## II. Instrument Characteristics

### A. Lasers

Lasers provide four major advantages as sources of excitation light : 1) a wide variety of excitation wave lengths; 2) a light beam that can be easily transported and shaped; 3) the possibility of narrow angle light scatter measurements; and 4) the light is monochromatic. Currently offered are argon, krypton, xenon, and helium-neon ion lasers and dye lasers. The latter are usually used with Rhodamine 6G as the dye. Table 1 and Fig. 1 show the wavelengths and powers available from several typical lasers. In some instances, such as for ultraviolet light, special optics are required and there is also usually a requirement for an increased total power input to the laser power supply. Lasers are inefficient at this wavelength and a laser with a total power of several watts is usually required for stable operation. Lasers can be obtained with very large amounts of power although current instruments are designed for a limit of about 12 watts. Disadvantages of lasers as excitation light sources are their size and cost, the latter including initial price, utilities and repair.

The manufacturer usually specifies the type and model laser that is incorporated into an instrument. This is necessary because lasers from different manufacturers have different physical sizes and shapes. However, since it is possible to modify any instrument, it can be useful to know what parameters are important in making a selection. The following are suggested, not in any particular order.

- 1) Total light output (all lines)
- 2) Light output at desired single wavelength lines
- 3) Ease of cleaning and changing mirrors
- 4) Variety of mirrors provided
- 5) AC Line Voltage
- 6) Maximum current required

- 7) Type of cooling
- 8) Water flow rate (gal/min) for water cooled lasers
- 9) Repair record (talk to other users)

## B. Arc lamps

In many flow cytometers, the excitation source consists of high pressure mercury and xenon arc lamps. These lamps are specified by the amount of power they consume rather than the power they emit. Most lamps in use are rated at 100 watts. Fig. 2 shows the light spectrum from a typical mercury lamp and Fig. 3 shows the spectrum of a xenon lamp. Optical filters are used to select the range of excitation wavelength desired. For example the UG1 excitation filter provides for illumination with 300-400 nm (uv) light. Total power provided by this combination is about 10mW. Two advantages of arc lamps over lasers as excitation light sources are: 1) they are much less expensive to purchase and to maintain; and 2) they are much smaller and easier to incorporate into instruments. Disadvantages include lower light output at all wavelengths, less selectivity of excitation wavelength (filters cannot produce monochromatic beams), more difficulty in shaping and transporting the beam, relatively short lifetimes for the lamp, instability of arc position and power, and no possibility for measuring light scatter at small angles.

The only option for a user in the selection of arc lamps is the type of tube; there is quite a variation in price and dependability. Other users are a good source of information.

## C. Flow Chambers

### 1) Flow Cytometers

Most flow chambers are constructed with square cross sections inside and outside, although a few are still available with a cylindrical cross section. The flat walls of the square cells

provide a better surface for optimum focussing of the laser beam and for collecting the fluorescent light. If imaging of the detection region is desired, the flat walls are required. The fluid path in these instruments is usually totally enclosed. An important point in this regard is to have a method of collecting all fluid that passes through the chamber, for safety purposes. Most fluorescent dyes that are used are either outright or potential carcinogens or mutagens and must be carefully handled and disposed of.

The different commercial instruments tend to have different fluidic and optical arrangements. The following paragraphs give brief descriptions of the general design of several of the instruments, to illustrate the variety available. Detailed design details are available from the manufacturers.

Fig. 4 is a schematic drawing of the FACS Analyzer (Becton Dickinson Immunocytometry Systems, Mountain View, California), showing the combination of fluidics and optics used in the instrument. Panel A shows the arrangement of the mercury arc lamp used in an epi illumination configuration. Panel B shows the internal flow cell arrangement that permits the measurement of fluorescence and cell volume simultaneously.

Fig. 5 shows a very different arrangement used by Kratel (Geneva, Switzerland) in their flow cytometer. In this instrument the flow is horizontal and includes a laser as the illumination source, in an epi illumination arrangement. It also uses a split laser beam and two cell intersection points to control the flow velocity.

Fig. 6 is a schematic drawing of the Skatron (Lier, Norway) flow cytometer. This instrument utilizes epi illumination for its fluorescence measurements and also measures forward angle light scatter. Its flow arrangement is a very novel one in that a nozzle is used to produce a flat, laminar flow across the open surface of a microscope cover glass, with the cells confined to a

narrow sector along the middle. The cells pass through the illumination beam as they flow across the cover slip.

## 2) Flow Sorters

In flow sorters, the fluid stream carrying the cells is forced to pass through a small orifice (e.g., a sapphire watch jewel with 80  $\mu\text{m}$  dia. hole), forming a fluid jet. Vibration of the chamber, also called the nozzle, results in the jet eventually breaking up into a stream of uniform droplets. In most instruments the drops are formed at a distance of about 6.5 mm from the orifice. In some sorters the laser beam used to excite the fluorescent dyes passes through the fluid stream shortly after it leaves the orifice. Measurements are made at this point. This is often referred to as the "sense in air" method. There is still sufficient time (about 150  $\mu\text{sec}$ ) to make a decision on whether to sort the cell, before the cell ends up in a drop. The advantage of this configuration is that this delay time is relatively short. For efficient sorting at high purity the time delay between cell detection and droplet charging must be very stable. Since no sorter is perfectly stable, the delay time should be kept as short as possible. Compensation for variation in delay time is made by sorting several drops for each cell. The major disadvantage for this type of detection/sorting arrangement is that the liquid stream acts as a very short focal length cylindrical lens. One result is that a large amount of the incident laser light is refracted and reflected into a plane of light that impinges onto the fluorescent light collection lens. To block this light a thin strip of metal (obscuration bar) is placed across the middle of the fluorescent light collection lens. This results in lowered light collection efficiency. Since the obscuration bar cannot block all of the scattered laser light, greater demands are placed on the optical filters placed in front of the light detectors (photomultipliers). The lens effect of the stream also makes it impossible to accurately image the cell/laser intersection point at the back focal point of the light collection lens. As a

result, the pin hole used to block unwanted light from impinging on the photomultipliers has to be larger than desired and can result in greater "noise" on the signal.

An alternate design utilizes the same type of square flow chamber as in a flow cytometer, with a watch jewel at the bottom. The laser beam passes through the flat walls of the chamber. The major advantages of this arrangement are 1) imaging of the measurement point is improved, 2) there is no plane of scattered laser light to increase background, 3) the light collection efficiency is higher since there is no obscuration bar, and 4) the flow velocity is lower, providing for more fluorescent light to improve measurement precision. Disadvantages are that the delay between cell detection and droplet charging is increased considerably, putting greater demands on flow stability, and a larger overall nozzle size, making the physical assembly larger.

Fig. 7 shows the design of the Becton Dickinson FACS 440 cell sorter. This sorter is of the "sense in air" design described earlier, where the laser beam goes through the fluid stream after it leaves the nozzle. Only one laser is shown in this diagram. The instrument is available with two lasers. For the second laser an additional pair of photomultipliers would be added, with the necessary electronics.

Fig. 8 shows the design of the Coulter (Hialeah, Florida) EPICS 700 series flow sorter. This is a dual laser arrangement which includes four detectors at 90 deg. and a forward scatter photodetector. In this sorter the laser beams intersect the flow stream in air.

Fig. 9 is a schematic diagram of the sorter manufactured by ODAM (Wissenbourg, France). It is a dual beam arrangement with three photomultipliers as fluorescence detectors. The light collection optics are unusual, utilizing a toroidal mirror assembly to obtain high fluorescent light collection efficiency.

Fig. 10 shows the arrangement used in the Partograph FMP Sorter manufactured by Kratel (Geneva, Switzerland). In this instrument, the measurement is made within a quartz cuvette. Laser excitation is made in an epi configuration, with forward and right angle detectors as well.

### III. Manufacturers

The following manufacturers, listed alphabetically, have contributed information to this chapter. For further or more detailed information on any of their products you are invited to contact them directly.

Becton Dickinson Immunocytometry Systems  
2375 Garcia Avenue \* P. O. Box 7375  
Mountain View, California 94039  
(800) 821 9796 in CA (800) 223 8226

Epics Division  
Coulter Corporation  
P. O. Box 4486  
Hialeah, Florida 33014-0486  
(305) 885-0131

Kratel, SA  
64 Ch. de St. Maurice/BP82  
CH-1222 Geneve-Vesenaz  
Switzerland (022)  
52 33 74

ODAM  
34, rue de L'Industrie  
67160 Wissenbourg  
France  
(88) 94.99.32

Skatron A/S

P.O.Box 8

N-3401 Lier

Norway

(03)850770

The following manufacturers provide partial systems that can be attached in one way or another to complete systems. In some instances, for example with data analysis systems, a flow cytometer or cell sorter can be purchased without a data analysis capability and one of the competitor computer systems purchased and added to the instrument. Some of the companies provide alternatives in the hardware, for example the Multichannel Analyzer.

A. Data acquisition/Analysis Systems

Brucker Spectrospin  
34, Rue de L'Industrie  
67160 Wissembourg  
FRANCE

Catalyss Corporation  
7400 South Tucson Way  
Englewood, Colorado 80112  
USA

Nuclear Data Incorporated  
Golf and Meacham Roads  
Schaumburg, Illinois 60196  
USA

B. Data Analysis Systems

Cellsoft Biotechnology Systems  
P.O. Box 13666  
Research Triangle Park, North Carolina 27709  
USA

Oatka Software  
P.O. Box 5  
Scottsville, New York 14546

Verity Software House, Incorporated  
P.O. Box 247  
Topsham, Maine 04086  
USA

Table 1

Power levels in watts for available monochromatic wavelengths for several lasers (a)

Wavelength (nm)	Argon 25 mw	Argon 5 watts	Argon 12 watts	Argon uv	Krypton 750 mw	He-Cd	He-Ne
325.0							.01
351.1-363.8		.060	.400	1.5	.075		
413.1					.060		
441.6							.05
454.5		.12	.60	.60			
457.9	.001	.35	.95	.95			
465.8		.20	.35	.35			
472.7		.30	.55	.55			
476.5	.002	.75	1.95	1.95			
488.0	.015	1.5	4.7	4.7			
496.5		.70	1.75	1.75			
514.5	.009	2.0	5.2	5.2			
528.7		.34	.80	.80			
632.8							.002
647.1					.500		
676.4					.120		
752.5					.100		

(a) The powers shown are for typical lasers. Some wavelengths require special optics. The different lasers are distinguished by total power for all lines. For more exact and detailed information please contact the manufacturers.

#### IV. Instrument Characteristics

##### Becton Dickinson Immunocytometry Systems

###### 1. FACS Analyzer

Excitation : Mercury arc lamp, 100 watts  
Detectors : Two fluorescence; one 90 deg. light scatter; volume sensor  
Flow chamber : 50-100 um dia.; 75-225 um long  
Sample conc. :  $10^5$  to  $2 \times 10^7$  cells/ml  
Data rate : 1,000/sec  
Resolution : 2% CV on fluorescence, 2% CV on volume  
Sensitivity : 8,000 molecules of fluorescein  
Signal proc. : log/lin amplifiers; ratios; electronic compensation; list mode data acquisition  
Data proc. : uses the Consort 30 data management system  
Utilities : 115 VAC, 15 amps

###### 2. FACS 440 Flow Sorter

Excitation : two argon ion lasers, 2 watts standard  
Detectors : four photomultipliers; one photo diode  
Flow chamber : sense in air  
Orifice : 50-200 um dia.  
Sample conc. :  $10^5$  to  $2 \times 10^7$  cells/ml  
Data rate : 10,000/sec  
Sort rate : 5,000/sec  
Resolution : 2% CV on fluorescence  
Sensitivity : 2,000 molecules of fluorescein  
Signal proc. : log/linear amps.; ratios; region of interest sorting; list mode data acquisition; polarization anisotropy; dual fluorescence compensation  
Data proc. : accumulates up to sixteen 256 channel histograms or one 64x64 channel histogram; display 1 or 2

univariate histograms or bivariate data in a 3d perspective view; area of interest integration

Utilities : 220 VAC, 50 amps, 5 gal/min water

Options : 5 or 6 watt argon and krypton ion lasers, including uv enhanced; single cell deposition into 96 well trays; micro sample delivery of 5-25 ul at .01-.05 ml/min; enclosed sensing region; dye laser; helium-neon laser; universal data lister

### 3. FACStar Flow Sorter

Excitation : 2 watt argon ion lasers

Detectors : forward scatter photodiodes; three photomultipliers for fluorescence and side scatter

Flow chamber : sense in air

Orifice : 50-200 um dia.

Sample conc. :  $10^5$  to  $2 \times 10^7$  cells/ml

Data rate : 5,000/sec

Sort rate : 7,000/sec

Sensitivity : 2,000 molecules fluorescein

Signal proc. : linear/log amplifiers, bivariate region of interest sorting with gating

Data proc. : Hewlett Packard Model 310 microcomputer

Utilities : 208 VAC at 50 amps, 5 gal/min water

Options : automated cloning; signal area and width calculation; quartz flow cell; 5 watt uv enhanced argon ion laser

### 4. Consort 30 Data Management System

CPU : HP 9920S, 640KB RAM memory

Storage : Dual floppy

Interaction : menu driven

Acquisition : univariate; bivariate; list mode

Data rate : 5,000/sec at four variables

Programming : HP BASIC and PASCAL 2.1

Display : histograms; dot plots; contour plots  
Analysis : region of interest calculation for gated data; DNA  
phase fraction analysis  
Options : 14.5 MB fixed disk

#### 5. Consort 40 Data Management System

CPU : LSI 11/23, 256KB RAM memory  
Storage : dual floppy  
Interaction : menu driven  
Acquisition : up to four variables in list mode, simultaneous sort  
control and analysis of four variables  
Data rate : 5,000/sec at four variables  
Programming : FORTRAN  
Display : multiple univariate plots; bivariate contour and  
perspective plots  
Analysis : uses LACEL (Los Alamos National Laboratory) software  
that permits non rectangular region of interest  
analysis of multiple gated bivariate distributions;  
programmable analysis sequence  
Options : up to 31.4MB fixed disks: graphics hard copy

#### 6. FACS Automate

Samples : 96 well trays  
Cycle time : 2 minutes between samples  
Volume : 5-50 ul/sample  
Rate : .08-.95 ul/sec  
Interfaces : FACS Analyzer and sorters

## 7. FACStar Plus

Excitation : Argon, Dye, UV-Argon, He-Ne, Krypton lasers  
 Detectors : six photodetectors, 8 variables in list mode  
 Flow chamber : sense-in-air, quartz cuvette optional  
 Orifice : 50-200  $\mu\text{m}$   
 Sample conc. :  $10^5$  to  $10^7$  cells/ml recommended  
 Data rate : event measurement time less than 30  $\mu\text{s}$   
 Sort rate : 7,000/sec  
 Sensitivity : 2,000 molecules of fluorescein per cell  
 Resolution : <2% on fluorescence  
 Signal proc. : lin/log amplifiers; pulse height, width and area  
 Data proc. : single and multiple histograms and contour plots;  
                   statistics; DNA curve fitting; histogram overlays;  
                   time mark on data  
 Utilities : 100/120VAC, 25 amps, or 220/240VAC, 12 amps; laser  
                   208VAC 3 phase, 50 amps; 5 gal/min water  
 Options : automatic sampling; automatic cell deposition;  
                   refrigeration; aerosol removal; closed flow cell  
  
 Computer : Hewlett Packard Model 310, 320; MicroVAX II  
 Storage : choice of floppy disks, hard disks, magtape  
 Interaction : menu-driven  
 Acquisition : eight variables, list mode  
 Programming : HP Pascal; DEC higher level languages  
 Display : dual oscilloscopes; computer CRT; real time dot  
                   plots, histograms, pulse display, contour plots  
 Options : mass storage devices; color graphics; printers;  
                   networking

## 8. FACScan

Excitation : 15mW Ar-ion laser  
 Detectors : 4 photomultipliers, 1 solid state silicon detector  
 Flow chamber : square quartz cuvette

Orifice : 430um by 180um  
Sample conc. :  $10^5$  to  $2 \times 10^7$  per ml  
Sensitivity : 1000 FITC molecules per particle  
Resolution : CV <2% for fluorescence  
Signal proc. : lin/log amplifiers; spectral overlap compensation  
Data proc. : single or multiple histograms, dot plots, contour plots; histogram overlays; non-parametric statistics; DNA analysis; immune monitoring software  
Utilities : 120 VAC, 20 amps  
Options : FACS AutoMATE automatic sample injection  
  
CPU : HP9000 Series, Model 310  
Storage : 20 Mbyte hard disk; microfloppy disk

### Coulter Corporation

#### 1. EPICS PROFILE Flow Cytometer

Excitation : 150 mw air cooled argon ion laser/ one watt argon ion laser/ or three watt argon ion laser  
Detectors : three photomultipliers, one photodiode  
Flow chamber : 250 um square quartz cuvette (125um optional)  
Sample conc. :  $5 \times 10^6$  cells per ml  
Data rate : 10,000 cells/sec  
Sensitivity : <1,000 molecules of fluorescein  
Signal proc. : One multiplexed successive approximation ADC with 1024 channel resolution; linear/log amplifiers; integral signals; signal overlap correction; amorphous gating windows; ratio of any two parameters; automatic computer control via test protocols  
Data proc. : high speed hardware data acquisition system; 32KB histogram memory; Intell 8088 16-bit microprocessor with 512 KB RAM memory; Intel 8087 floating point co-processor; 64-256-1024 channel histograms;; quad

display screen; contour and dot plots; numerical and statistical analysis; automatic computer driven test protocols

Storage : one 5 1/4" floppy disk drive with 360KB storage; one 20MB hard disk

Utilities : 150mw laser 110 VAC, 15 amps, air cooled  
 1000mw laser 208 VAC, single phase/60  
 amps, 1.9 gal/min water  
 3000mw laser 208 VAC, 3 phase/40 amps,  
 2.2 gal/min water

Options : streaming magnetic tape

## 2. EPICS CS Flow Sorter

Excitation : two watt argon ion laser (five watt optional)

Detectors : three photomultipliers, one photodiode

Flow chamber : sense in air standard (quartz flow cell optional)

Orifice : 76um dia. (50um to 250 um optional)

Sample conc. :  $5 \times 10^6$ /ml

Data rate : 10,000/sec

Sort rate : 5,000/sec

Sensitivity : <1,000 molecules of fluorescein

Signal proc. : four ADC's; linear/log amplifiers; peak or integral signal; signal overlap correction; ratios of any 2 signals; 16 sided gating window; list mode; computer control via test protocols

Data proc. : high speed hardware data acquisition system; 32KB histogram memory; Intel 8086 16-bit microprocessor with 512 KB RAM memory; Intel 8087 floating point coprocessor; 4 histograms per measurement; 64-256-1024 channel histograms; quad display screen, 3d perspective views of bivariate data; gated bivariate displays; contour and dot plots; nonparametric

analysis of univariate data; numerical statistical analysis

Storage : one 8" floppy disk drive, 1MB storage per disk; one 20MB hard disk drive

Utilities : 208 VAC, 50 amps, 2.2 gal/min water

Options : Coulter volume; Autoclone; microsampler delivery system; biohazard control system; upgrade to Model CD sorter; streaming tape drive; EASY 88 data analysis system; cytologic software

### 3. EPICS CD Flow Sorter

Same as EPICS CS, with dual laser bench for addition of second laser. Three photomultipliers, 2 photodiodes

### 4. EPICS 541 Flow Sorter

Excitation : two watt argon ion laser (five watt optional)

Detectors : three photomultipliers, one photodiode

Flow chamber : sense-in-air standard (quartz flow cell optional)

Orifice : 76um dia. standard (50um to 250 um optional)

Sample conc. :  $5 \times 10^6$  cells/ml

Data rate : 10,000/sec

Sort rate : 5,000/sec

Sensitivity : <1,000 molecules of fluorescein

Signal proc. : 4 ADC's; linear/log amplifiers; signal amplitude or area; time; list mode; 16 sided gating window; signal overlap correction

Data proc. : Intel 8085 microprocessor data acquisition system; 32 KB histogram memory; Intel 8086 microprocessor with 8087 floating point co-processor for online analysis with 512 KB RAM memory; up to 8 histograms per run; quad display screen; bivariate dot plots with windowing; 3D display of bivariate data; numerical statistical analysis; acquisition

protocols; nonparametric analysis of univariate data

Storage : two 8" floppy disk drives with 1MB per disk

Utilities : 208 VAC, 50 amps, 2.2 gal/min water

Options : 2 additional ADC's; 1024 channel ADC's; emission anisotropy; polarization; time of flight; ratios of any two signals; 20MB or 40MB hard disk; microsampler delivery system; auto-clone; auto sort lock; biohazard control system; Coulter volume adapter; streaming tape drive; EASY 88 data analysis system; cytologic software

## 5. EPICS 751 sorter

Excitation : 5 watt argon ion laser

Detectors : 4 photomultipliers, 1 photodiode

Flow chamber : sense in air standard (quartz flow cell optional)

Orifice : 76 um dia. standard (50um to 250 um optional)

Sample conc. :  $5 \times 10^6$ /ml

Data rate : 10,000/sec

Sort rate : 5,000/sec

Sensitivity : <1,000 molecules fluorescein

Signal proc. : 6 ADC's; linear/log amplifiers; pulse height and area; time of flight; polarization; emission anisotropy; 16 sided gating window; list mode; ratios of any two signals; signal overlap correction; time; microprocessor controlled

Data proc. : Intel 8085 microprocessor data acquisition system; 32KB histogram memory; Intel 8086 microprocessor with 8087 floating point co-processor for online analysis with 512KB RAM memory; up to 8 histograms per run; quad display screen; bivariate dot plots with windowing; 3d display of bivariate data; numerical statistical analysis; acquisition protocols; nonparametric analysis of univariate data

Storage : one 8" floppy disk drive with 1MB storage; 20MB hard disk

Utilities : 208 VAC, 50 amps, 2.2 gal/min water  
Options : Microsample Delivery System; Auto-Clone System; EASY  
88 data analysis system; 40MB hard disk; Biohazard  
Control System; Coulter Volume Adapter; Auto Sort  
Lock; streaming tape drive; cytologic software

#### 6. EPICS 752 Flow Sorter

This sorter has the same features as the Model 751, with the addition of a dye laser to add more capability in the use of more than one fluorescent dye. The argon laser, operating in an all-lines mode, provides a 488nm beam for direct cell excitation and a 514 nm beam for pumping the dye laser. The most frequently used dye is Rhodamine 6G. PRISM parameter standard for multi-color real time analysis and sorting.

#### 7. EPICS 753 Flow Sorter

This sorter has the same features as the Model 752, with the addition of a second argon ion laser to pump the dye laser. The second laser also provides for simultaneous operation of visible and uv beams.

#### 8. Auto-Clone

A programmable automatic single-cell deposition system that sorts from 1 to 10 cells or multiples of 100 cells per well into multiwell microculture plates.

Samples : 24, 60, 96 well trays  
Cells/well : 1 to 10 per well, or multiples of 100  
Timing : 2 sec per well, 3.2 min for 96 well tray

#### 9. Coulter Volume Adapter (No sorting)

The EPICS Coulter Volume Adapter (CVA) is an accessory that allows the volumetric measurement of cells and other particles using the electrical impedance method known as the Coulter Principle.

Aperture : 50x50x50 um  
Current : 50-500 uA  
Dia. range : 1-17 um dia.  
Flow rate : 0-40 ul/min

#### 10. Auto Sort Lock

This option, available for the 750 series of sorters, stabilizes the droplet breakoff point to a precision of +/- 0.1 drops. It also includes ultrasonic excitation to help remove plugs in the orifice, controls the number of drops sorted and calculates the sort matrix for 1, 2 or 3 drop sorting.

#### 11. Microsample Delivery System

The MicroSampler Delivery System (MSDS) provides automated sample introduction and data acquisition for both single samples via standard 12 x 75mm tubes and multiple samples via 96-well microtiter plates.

Samples : 96 well microtiter trays and 12 x 75mm tubes, with temperature control (5+/-1 deg C), includes mix and wash cycles, selective sampling  
Cycle time : variable, depending on sample and protocol  
Volume : 10-100 ul/sample  
Rate : 10-250 ul/min delivery  
Interfaces : all EPICS sorters

## 12. Biohazard Containment System

The EPICS Biohazard Containment System (BCD) provides a totally enclosed environment for sample processing which eliminates aerosols from hazardous materials. The system consists of a biohazard containment drawer (BCD) and a 250 um square quartz flow cell for analysis only. The drawer houses a 3 liter waste container that can be incinerated for disposal.

## 13. EASY 88 Data Analysis System

CPU	: Intel 8088 with Intel 8087 floating point co-processor, MS-DOS operating system, Model 7220 graphics microprocessor
Storage	: 20MB hard disk, 10MB tape cassette
Interaction	: Menu system
Acquisition	: Serial and parallel communications channels, EPINET network system
Programming	: Can run most commercial software written for use with the MS-DOS operating system
Display	: A 13" color monitor with 640 x 480 pixel resolution, with log or linear scaling of the y axis, zoom-in on specific areas of the data
Analysis	: EASY 88 provides a wide variety of numerical, statistical and graphical methods of analyzing data. Included are methods of comparing two or more histograms (graphical and statistical), list mode processing to produce multiple gated histograms, DNA phase fraction analysis
Options	: 20 MB hard disk, EPINET data links to sorters, floppy disk drives

#### 14. Cytologic

Cytologic software is specifically designed for flow cytometry data analysis and graphics. It is compatible with IBM PC, XT, AT and equivalent systems. Written in Microsoft BASIC, it allows individuals to compile and link their own BASIC applications programs to the Cytologic package.

Two packages are available:

Cytologic Software for Immunofluorescence Applications

Cytologic software for DNA Applications

#### Kratel SA

##### 1. Partograph FMP Analyzer

Excitation	: Argon ion and He-Ne, dual beam
Detectors	: 2 photomultipliers, 2 photodiodes
Flow chamber	: quartz flow cell, 250 x 250 um
Sample conc.	: $10^7$ /ml
Data rate	: 10,000/sec
Sensitivity	: 3,000 molecules of fluorescein
Signal proc.	: log/linear amplifiers, pulse height, length and area, flow velocity
Data proc.	: microprocessor controlled (Z80) acquisition of all 4 signals, dual floppy disk system with BASIC and FORTRAN programming, real time graphical display with contour and 3d perspective plots of bivariate data
Utilities	: 220 VAC, 10 amps
Options	: mercury arc lamp, higher powered lasers, autosampler

##### 2. CYTOMIC 12

CPU : Z 80 microprocessor, 20KB ROM, 36KB RAM memory  
Storage : 74 univariate distributions of 128 channels, or 3  
bivariate distributions of 4096 channels each  
Interaction : 20 key keyboard  
Acquisition : one or two variables, with gating  
Display : univariate distributions, with overlay of a second;  
bivariate data as contour or isometric plots, the  
latter with rotation  
Analysis :  
Options : floppy disk, holding 4800 univariate  
distributions or 132 bivariate distributions;  
printer

### 3. Partograph FMP Sorter

Excitation : Argon ion and He-Ne, dual beam  
Detectors : 2 photomultipliers, 2 photodiodes  
Flow chamber : quartz flow cell, 250 x 250  $\mu\text{m}$ , with 70  $\mu\text{m}$  orifice  
Sample conc. :  $10^7/\text{ml}$   
Data rate : 10,000/sec  
Sort rate : 3,000/sec  
Sensitivity : 3,000 molecules of fluorescein  
Signal proc. : log/linear amplifiers, pulse height, length and  
area, flow velocity  
Data proc. : microprocessor controlled (IBM PC/AT 2) acquisition  
of all 4 signals, dual floppy disk system with BASIC  
and FORTRAN programming, hard disk, real time  
graphical display with contour and 3d perspective  
plots of bivariate data  
Utilities : 220 VAC, 10 amps  
Options : mercury arc lamp, higher powered lasers, autosampler

ODAM

1. ATC 3000

Excitation : Argon and Krypton ion lasers, dye lasers, dual system

Detectors : 3 photomultipliers, 1 photodiode, 1 electrical cell volume detector, multiangle light collection optics

Flow chamber : sense in a quartz cell, with 2 sheaths

Orifice : 70 um to 120 um dia

Sample :  $5 \times 10^6$ /ml, with sample agitation and temperature control

Data rate : 10,000/sec

Sort rate : 5,000/sec

Sensitivity : 2,000 molecules of fluorescein

Resolution : 0.8% on fluorescence, 1.1% on scatter, 3% on volume

Signal proc. : Intel 8085 analytical adjustments control processor, log/linear amplifiers, ratios, up to 8 measured and derived parameters are transferred into an 8 input ADC

Data proc. : online processing detailed below

Utilities : 380 VAC, 50 Hz, 70 amps for dual laser configuration; 18 liters per min cooling water

Options : single cell deposition

  

CPU : 24 bit bit-sliced central processer unit (AMD 2900)

Storage : hard disk with 32, 80 or 160 MB capacity, and 512KB floppy

Interaction : programmable

Acquisition : 8 univariate and 8 bivariate distributions in realtime and list mode, 8 histograms with 256 channels per histogram, 8 cytograms with 256x256 points per cytogram

Display : two graphics coprocessors, two color monitors, and one ASCII text display, dot plots, contour plots, 3d perspective views

- Analysis** : 10 region of interest areas of any shape, (10 cursors in histograms, 10 windows in cytograms), data (curve) smoothing, comparison, subtraction, mode and CV calculation, calculation of cell cycle phase fractions, overlay of 3 histograms, specialized programs dealing with chromosome analysis and immunology, user programmability in PASCAL
- Options** : 18MB magnetic tape storage unit, black and white ink jet printer, color ink jet printer, ethernet interface

Skatron A/S

1. Argus Flow Microphotometer

Excitation : 100 W Hg arc lamp, air cooled Ar-laser optional  
Detectors : 5 photomultipliers, for fluorescence and light  
scatter  
Flow chamber : Jet On Open Surface (J00S) type  
Orifice : 70 or 100  $\mu\text{m}$   
Sample conc. : Up to  $10^9$  per ml  
Data rate :  $10^4$  per sec  
Resolution : Fluorescence: CV=1%, Light scatter: CV=0.6%  
Signal proc. : Lin/Log amplifiers, electronic compensation  
Data proc. : Accumulates four 256 channel histograms and  
three 64x64 channel histograms simultaneously,  
plus list mode  
Data proc. : Region of interest, integrate, mode, CV,  
Bivariate to univariate histogram collapse  
Utilities : 240 VAC or 110 VAC

Illustrations

Figure 1 Output power for wavelengths available from 12 Watt argon, 759 mW krypton, and small He-Cd and He-Ne ion lasers. Refer to Table 1 for precise wavelengths.

Figure 2 Relative intensity vs wavelength for the emission from a 100 watt mercury arc lamp.

Figure 3 Relative intensity vs wavelength for the emission from a 100 watt xenon arc lamp.

Figure 4 A. Optical path of the Becton Dickinson FACS Analyzer.  
B. Fluidics path for the FACS Analyzer.

Figure 5 Optical/fluidic arrangement of the flow cytometer produced by Kratel SA.

Figure 6 Schematic drawing of the microscope based flow cytometer manufactured by Skatron A/S.

Figure 7 Schematic drawing of the Becton Dickinson FACS 440 flow sorter.

Figure 8 Schematic drawing of the Coulter EPICS 750 series flow sorter.

Figure 9 Schematic drawing of the ODAM flow sorter.

Figure 10 Schematic drawing of the Kratel Partograph FMP Sorter.





















